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Evaluation of Four Reagents for Delipidation of Serum

S. T. AGNESE, F. W. SPIERTO,[1] and W. HARRY HANNON

The Centers for Disease Control, Public Health Service, Department of Health and Human Services, Atlanta, Georgia 30333

Four reagents, Aerosil 380, Freon 113, Dextran sulfate 500-S, and a mixed organic solvent were tested for their abilities to produce optically clear, pooled human serum. Aerosil-380, a silicon dioxide, removed 95% of serum cholesterol and triglycerides, and 80% of the free fatty acids. A mixed organic solvent (*n*-butanol:diisopropyl ether) was equally effective, but also removed nearly all endogenous alkaline phosphatase and lactate dehydrogenase. Freon-113 and Dextran sulfate 500-S removed about half of the serum cholesterol and triglycerides. The serum content of several non-lipid components was unaffected by Aerosil-380, Freon-113, and Dextran sulfate treatments; however, the mixed organic solvent removed 69% of the endogenous calcium. Light scattering data revealed that treatment with all reagents except the mixed organic solvent resulted in optically-clear serum products.

Reference sera are needed for quality control and as secondary calibration standards for the clinical chemistry laboratory. They should be optically clear, sterile and chemically similar to normal human serum. Lyophilized materials should be easily reconstituted and provide an optically clear solution.

Several investigators have reported that optically-clear serum pools can be produced using methods which remove serum lipids. Proksch and Bonderman (1) showed that turbid serum could be clarified by treatement with dextran sulfate. Chemical analysis revealed that the only changes this treatment produced were 75% reduction of cholesterol and triglycerides. Reimer *et al.* (2) also described a delipidation procedure with Freon-113, a trifluoro-trichloro-ethane, for producing sera which were found to be transparent when examined by light-scattering methods. Cham and Knowles (3) found that treatment of serum with an equal volume of a 40:60 (V:V) butanol-diisopropyl ether mixture produced a lipid-free serum pool containing normal levels of proteins, enzyme activities, and small molecules. Other investigations (4) revealed that treatment with Aerosil-380, a colloidal silica gel, greatly increased the titer of certain arbovirus antigens, possibly due to the removal of lipoprotein inhibitory factors.

We undertook this study in order to compare the effects of various delipidation reagents on the chemical composition and optical clarity of various analytes in pooled, normal human serum.

Materials and methods

An Omni mixing apparatus (E. I. DuPont Co., Wilmington, DE 19898) was used to extract serum with Freon. A Sorvall RC2-B Centrifuge with type GSA rotor (E. I. DuPont Co., Newton, CT 06470) was used for centrifugation. We filtered serum and serum extracts with equipment from the Millipore Corporation (Bedford, MA 01730). We also used: Freon 113 ($Cl_2CFCClF_2$ – from E. I. DuPont Co.), Aerosil 380 (Degussa Inc., Teterboro, NJ 07608), Dextran sulfate 500-S (Sigma Chemical Co., St. Louis, MO 63178), diatomaceous filter aid and Kaolin (Johns-Manville, Co., New York, NY 10020). All other commercially available chemicals were of Reagent Grade.

Serum pools

Three human serum pools with artificially adjusted levels of cholesterol, triglyceride, and free fatty acids were prepared by methods described by Williams *et al.* (5). Serum pH was brought to 7.5 with small volumes of 0.5 mol/L acetic acid or 2.0 mol/L sodium bicarbonate. Any pooled serum showing excessive turbidity or particulate matter was pretreated with diatomaceous filter aid. Approximately 4 g was added to one L of serum, the mixture gently agitated for 15 min, then allowed to stand at ambient temperature for 1 h. Particulate matter was removed by centrifugation at 5000 rpm at 4°C for 20 min.

Delipidation procedures

1. Freon-113 treatment

Equal volumes of serum and Freon-113 were vigorously blended with the Omni mixer for 30 min. The mixture was transferred to a 500 mL separatory funnel, the denser Freon phase was discarded, and the extraction repeated. This extract was centrifuged (2000 RPM) for 90 min at 20°C. Residual Freon was removed after freezing and thawing the serum supernatant twice during a 48 h period. The final product was produced by filtration through 0.22 micron filters. The volume of serum recovered was 90%.

2. Dextran sulfate 500-S treatment

Thirty mg of dextran sulfate 500-S suspended in 2 mL H_2O was added to a 250 mL polycarbonate flat-bottom centrifuge bottle containing 100 mL of serum. The suspension was gently mixed 5 min, left to stand for 60 min, then centrifuged (10°C, 20 min) at 2000 RPM. The clarified serum supernatant was filtered through a 0.22 micron filter. Volume recovered was 99%.

3. Butanol-diisopropyl ether treatment

One hundred mL of serum, 10 mg EDTA and 150 mL of a solvent containing butanol and diisopropyl ether (40:60, V:V) were mixed for 10 min in a 500 mL

[1]Correspondence to: F. W. Spierto, Centers for Disease Control, 1600 Clifton Road, Atlanta, Georgia 30333.

TABLE 1
Effect of Various Treatments on the Removal of Lipids from Three Human Serum Pools

Analyte and Pool	Initial Content	Percentage Removed By			
		Solvent	Aerosil-380	Freon-113	Dextran Sulfate-500S
Cholesterol (mg/L)					
Pool-1	3030	98	95	76	48
Pool-2	2380	98	100	60	40
Pool-3	1640	91	98	82	51
Triglycerides (mg/L)					
Pool-1	1460	100	90	81	51
Pool-2	1130	100	100	59	40
Pool-3	750	95	100	83	53
Fatty Acids (mmol/L)					
Pool-1	2.79	81	17	10	16
Pool-2	2.26	80	34	22	22
Pool-3	1.49	73	15	39	39

TABLE 2
Effect of Various Treatments on the Removal of Selected Proteins and Small Molecular Mass Substances from 3 Human Serum Pools (Average or Range)

Analyte	Percent Removed by Treatment[a]			
	Solvent	Aerosil-380	Freon-113	Dextran Sulfate-500S
Alkaline Phosphatase (IU/litre)	86 to 97	2 to 6	−4	−4
Lactate Dehydrogenase (IU/litre)	82 to 100	3 to 15	−30	−29
Aspartate Transaminase (IU/litre)	32 to 67	59 to 70	60	64
Total Protein (g/litre)	−14 to −10	0 to 6	−2	−2
Albumin (g/litre)	−19 to −21	−2 to 7	−5	2
Calcium (mg/litre)	69	2 to 5	3	−1
Blood Urea Nitrogen (mg/litre)	0	0	0	0
Phosphorus (mg/litre)	−3	0 to 9	12	7
Potassium (meq/litre)	−10	1 to 4	−6	−4
Uric Acid (mg/litre)	−10	−6 to −4	10	2
Chloride (meq/litre)	−12	−2 to 2	−4	0
Sodium (meq/litre)	−10	−6 to 0	1	−1

[a]Negative numbers indicate an increase over initial content of a particular analyte.

Erlenmeyer flask under a fume hood. The mixture was transferred to a 500-mL separatory funnel, and of the three phases which separated, the uppermost was discarded. Two volumes of diisopropyl ether were added to the remaining liquid and gently mixed for 2 min. After phase separation, the delipidated serum was drained into a 250 mL glass centrifuge bottle and centrifuged (2000 RPM) for 45 min at 10°C. Most of the residual solvent from the upper phase was removed with a Pasteur pipet, and the remainder under vacuum. The delipidated serum was frozen, then thawed over a 48 h period. The product was filtered with difficulty using a 0.45 micron filter, yielding 75–85 mL of a cloudy suspension.

TABLE 3
Light Scattering Produced by Untreated and Delipidated Normal Human Serum[a]

Treatment	Pool I	Pool II	Pool III
None	60	48	30
Freon-113	18	21	7
Dextran sulfate 500-S	21	24	20
Aerosil-380	35	29	16
Solvent	78	54	50

[a]Nephelometer was set at zero using glass-distilled, deionized water.

4. Aerosil-380 treatment

Two g Aerosil-380 were added to 100 mL serum and the suspension was stirred for 2 h. Aerosil was removed by centrifugation (10,000 RPM) for 30 min at 10°C. The decanted serum was easily filtered through a coarse filter, then, with difficulty through 1.2 and 0.45 micron filters. Recovery was 95%.

CHEMICAL ANALYSIS

Two mL aliquots of serum and serum extracts were transferred to 2.5 mL Wheaton vials, capped, sealed, and frozen at −70°C until analyzed. Most chemical determinations were made with a Technicon SMAC® system. Triglycerides, cholesterol and free fatty acids were assayed by published procedures (Refs. 8, 9, and 10, respectively). Optical clarity was evaluated by nephelometry (2).

Results

Table 1 shows the effects of the described procedures the lipid composition of the three serum pools. The mixed organic solvent and Aerosil-380 removed nearly all cholesterol and triglycerides, whereas Freon removed approximately 75%, and dextran sulfate only 50%. About 80% of the nonesterified fatty acids was removed by the mixed solvent treatment, but only small amounts by the other procedures. Electrophoretic examination showed that Aerosil-380 removed all of the alpha and beta lipoproteins, and dextran sulfate removed approximately half of the beta-lipoprotein.

As expected, the organic solvent removed all serum lipids, but also inactivated alkaline phosphatase, lactate dehydrogenase, and aspartate transaminase (AST) (Table 2). AST activities were also considerably reduced by the three other treatments. The total protein and albumin content showed no change except with solvent treatment which resulted in an apparent increase. The mixed organic reagent removed 69% of the serum Ca.

The effect of the various delipidation procedures on optical clarity is shown in Table 3. By measuring light scattering intensities of 1:40 dilutions of sera before and after treatment, it was possible to relate optical clarity to treatment. It can be seen that all three serum pools produced less scattered light after Aerosil, Freon, and dextran sulfate treatments. Serum treated with the mixed organic solvent showed increased turbidity.

Discussion

Our major intent was to produce sera which were optically clear and chemically similar to untreated sera. We found that 3 of the 4 treatments accomplished this. We prefer using Aerosil-380 or Dextran sulfate because they can be easily removed from serum. On the other hand, Freon-113 and the mixed organic solvent are more difficult to remove. We cannot recommend use of the mixed organic solvent, primarily because it produces serum pools which are more cloudy than untreated specimens (Table 3). It also causes a substantial reduction in Ca, alkaline phosphatase, and total protein levels. The three other treatments produce optically clear products (Table 3). Although they, like the mixed organic solvent, also inactivate Aspartate Transaminase, they cause little change in the levels of Alkaline Phosphatase, Total Protein, Albumin, Ca, BUN, Inorganic Phosphorus, K, Uric Acid, Na, and Cl.

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